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Denitrification and Ammonia Formation in Anaerobic Coastal Sediments

ISAO KOIKE AND AKIHIKO HATTORI*
Ocean Research Institute, University of Tokyo, Nakano, Tokyo 164, Japan

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Simultaneous determinations of nitrogen gas production, ammonia, and particulate organic nitrogen formation in the coastal sediments of Mangoku-Ura, Simoda Bay, and Tokyo Bay were made by using the ^{15}N-label tracer method. The rate of nitrogen gas production in the sediment surface layer was about 10^{-2} μg atom of N per g per h, irrespective of the location of the sediments examined. [^{15}N]ammonia and -particulate organic nitrogen accounted for 20 to 70% of the three products, and after several hours of incubation, the major fraction of nondenitrified ^{15}N in Mangoku-Ura and Simoda Bay sediments was recovered as ammonia. In Tokyo Bay sediments, particulate organic nitrogen was produced at a greater rate than was ammonia. The reduction rate data suggest that the pathway of nitrate reduction to ammonia is important in coastal sediments.

Transformation of nitrogen and its recycling in coastal sediments contribute significantly to eutrophication in coastal areas. Denitrification, the reduction of nitrate and nitrite to gaseous nitrogen, and its significance in the nitrogen budget of lakes have been investigated by Chen et al. (3) and Keeney (9). Goering and Pamatmat (6) demonstrated the occurrence of denitrification in the marine sediments off the coast of Peru. However, little is known about the quantitative aspects of denitrification in marine sediments.

Microbial reduction of nitrate can be divided into two categories: assimilatory and dissimilatory (11). The product of assimilatory nitrate reduction is ammonia that is incorporated into cell material. Denitrification is a type of dissimilatory nitrate reduction that is responsible for loss of combined nitrogen from ecosystems. Lowered oxygen tension is required for dissimilatory nitrate reduction to proceed. But, even under these conditions, some bacteria including enteric bacteria produce ammonia (8).

This paper reports the simultaneous determination of N_2, ammonia, and particulate organic nitrogen (PON) produced in coastal sediments by using ^{15}N-labeled compounds.

MATERIALS AND METHODS

Sediment sampling. Sediment samples were collected from deep sediments with a Phleger corer. Divers collected samples from shallow sediments with plastic tubes. Sediment characteristics are described in Table 1. The organic nitrogen contents ranged from 0.56 to 7.8 mg of N per g. The water above the sediment in Tokyo Bay was anoxic, but in the other areas studied it contained oxygen. The presence of H_2S in all sediments suggested that they were anoxic.

Experimental procedures. Within a few hours after sampling, samples of sediment (about 1 g, wet weight) were placed in vacuum-tight incubation flasks (34 ml), which contained one-way stopcocks and rubber stoppers. Unless otherwise stated, samples of sediment from 0 to 3 cm were used. Sterile seawater (30 ml) containing less than 0.5 μg atom of nitrate- and nitrite-N per liter was added together with [^{15}N]nitrate or -nitrite (50% ^{15}N). The flasks were flushed with N_2 for about 7 min to remove oxygen, filled with sterile seawater saturated with N_2, and incubated at in situ temperature. Final concentration of added [^{15}N]nitrate or -nitrite was 15 μg atom of N per liter in the experimental sediments from Simoda Bay and Tokyo Bay and 30 μg atom of N per liter in that from Mangoku-Ura. After defined periods of incubation, the biological reactions were stopped by introducing 1 ml of 0.1 M $HgCl_2$ with a hypodermic needle through the rubber stopper. Samples treated with $HgCl_2$ immediately after the addition of [^{15}N]nitrate or -nitrite served as controls.

Analysis of ^{15}N in nitrogen gas. Incubation flasks were connected to gas samplers (200 ml) with a flexible tube and then evacuated to less than 10^{-3} mm of Hg. After evacuation the dissolved gases were extracted and collected in the gas sampler. More than 99% of the dissolved N_2 was extracted by this procedure. To eliminate water and CO_2, the gas samplers were cooled by dipping in liquid nitrogen for 15 min. After water and CO_2 removal, the nitrogen was introduced into the inlet system of a mass spectrometer, fitted with a CuO furnace (400°C), a Cu furnace (700°C), and a liquid nitrogen trap. The purified nitrogen was collected on a molecular sieve (Shimazu 5A) chilled with liquid nitrogen and was then dispersed into a defined volume by heating it to 180°C. Details of the inlet system and the determination of nitrogen gas concentration are described by Wada et al. (16). ^{15}N content

was determined with a Hitachi RMU-6 mass spectrometer fitted with a double collector and a dual inlet system for ratiometry. N_2 production from nitrate or nitrite was calculated by the method of Hauck et al. (7).

Analysis of ^{15}N in ammonia and PON. After gas extraction, the material in the incubation flasks was filtered through a Yumicron membrane filter (Yuasa Battery Co., Tokyo; average pore size, 0.4 μm). Ammonia adsorbed on the sediment was removed by washing the filter twice with 10 ml of 2 M KCl. The filtrate and the KCl washes were combined. Ammonia was separated and collected in 5 ml of 0.01 N HCl by steam distillation by the method of Bremner and Keeney (2). The quantity of ammonia in samples of the distillate was determined by the Sagi method (13). The ammonia in the remaining distillate was oxidized to N_2 with KOBr (12).

The residual sediment on the filter was washed with 30 ml of 3% NaCl to remove nitrate and nitrite, dried at 60°C, and weighed. A sample of the dried sediment was digested by a modification of the Dumas method (16), and the organic nitrogen was converted to N_2. The ^{15}N content of the N_2 was determined as described previously. Ammonia and PON production from nitrate or nitrite were calculated from the ^{15}N content and amounts of ammonia and PON and expressed in terms of μg atom of N per g (dry weight) per h.

Bacteriological methods. Viable numbers of heterotrophic, nitrite-producing and denitrifying bacteria in the sediments were approximated by the most-probable-number method (10). The numbers are given on a dry-weight basis.

RESULTS

N_2 production from nitrate and nitrite in the surface sediments proceeded almost linearly with time during the first 8 to 10 h (Fig. 1). Enzyme systems for nitrate reduction are known to be derepressible (11). This is especially true of denitrifying enzymes. However, except for N_2 production from nitrite in Tokyo Bay sediment, the derepression phase, which was observed for bacteria in water from the anoxic layer of a brackish lake (10), was not observed. This suggests that the enzymes required for in situ dissimilatory nitrate reduction were already present. The sediments had the conditions nec-

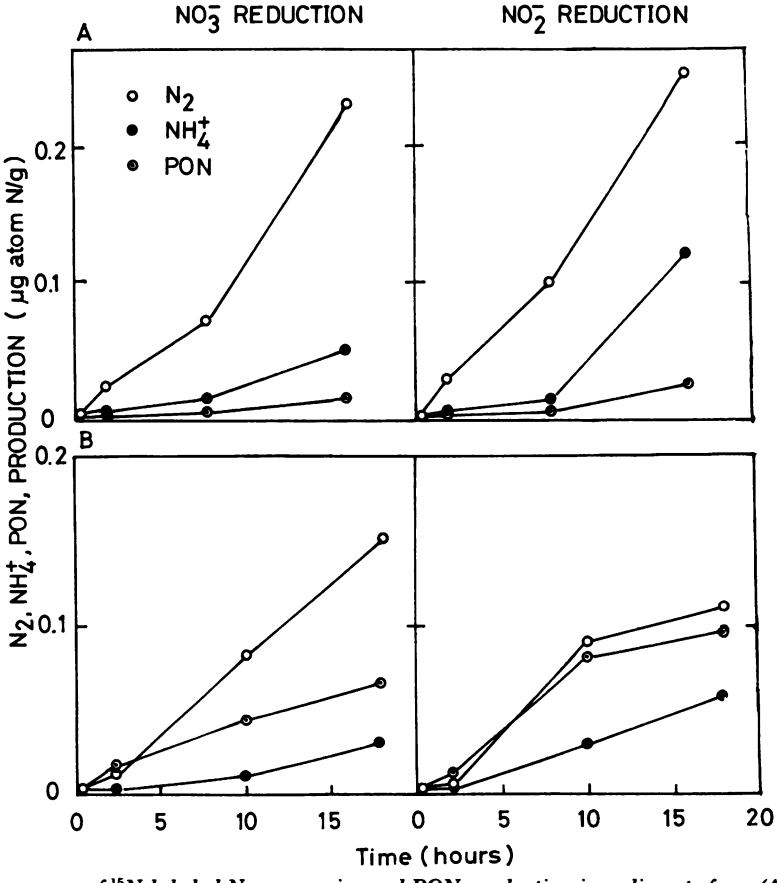

FIG. 1. *Time course of ^{15}N-labeled N_2, ammonia, and PON production in sediments from (A) Simoda Bay and (B) Tokyo Bay. Experimental conditions were the same as those described in footnote a of Table 2.*

essary to sustain dissimilatory reduction of nitrate, i.e., anaerobiosis and a supply of organic matter (reductant) (Table 1).

Besides N_2 production, nitrate (nitrite) was further reduced to ammonia and organic nitrogen (Fig. 1). However, the extent of these reactions varied depending upon the types of sediment (Table 2). An increased ^{15}N incorporation into ammonia or PON during the later period of incubation was occasionally observed. This can be attributed to an increase in bacterial biomass during prolonged incubation.

The rate of ammonia and PON production were roughly correlated with the organic content of the sediments. In the sediments of Simoda Bay and Tokyo Bay, denitrification formed the main path of anaerobic reduction on nitrate. However, in Mangoku-Ura sediments, which were richer in organic content (Table 1), ammonia and PON accounted for 52 and 15% of products of nitrate reduction, respectively. Ammonia production was three to four times faster than PON production by the microflora in Mangoku-Ura and Simoda Bay sediments. But, PON production was five times faster than ammonia production in the microflora in Tokyo Bay sediment.

In microbial systems, nitrate is reduced to nitrogen gas or ammonia via nitrite (11). In our sediment samples, the rate of N_2 production from nitrite was almost identical to that from nitrate (Fig. 1). This suggests that the reduction of nitrite to nitrogen gas or ammonia is rate limiting. The number of bacteria capable of reducing nitrate to nitrite was two orders of magnitude larger than that of denitrifying bacteria (Table 3). This result was consistent with the activity data. Population densities of denitrifying bacteria decreased with the depth of sediment. The denitrification activity likewise decreased with depth (Table 3).

DISCUSSION

The denitrifying activity of bacteria in coastal sediments is approximately proportional to nitrate concentrations in the range of 0 to 30 μg atom of N per liter (I. Koike, A. Hattori, and J. J. Goering, Mar. Sci. Comm., in press). The rate of N_2 production at a nitrate concentration of 15 μg atom of N per liter was calculated from the values in Table 2 to be 1.5×10^{-2} μg atom of N per g per h for Mangoku-Ura sediment, 1.8×10^{-2} for Simoda Bay sediment, and 8.4×10^{-3} for Tokyo Bay sediment. The denitrification activities of the three sediments are similar, although the type and organic contents of the sediments are considerably different (Table 1).

Owing to technical difficulties, we did not follow ^{15}N incorporation from [^{15}N]nitrate ([^{15}N]nitrite) into nitrogen oxides (nitric oxide and nitrous oxide) and dissolved organic nitrogen. Nitric and nitrous oxides are known to be produced as intermediates in bacterial denitrification (11). However, nitric oxide is a minor product, and its contribution can be safely disregarded. According to Wijler and Delwiche (17), nitrous oxide production in soil systems varies with pH. The higher the pH, the lower the production of nitrous oxide. N_2 is the main product of soil denitrification at pH 8.0, and the production of nitrous oxide is negligibly low (15). The pH of coastal sediment is about 7.5 or higher

TABLE 1. *Characteristics of coastal sediments (0 to 3 cm) used in the experiments*

Location	Sampling date	Depth (m)	Temp (°C)	Texture	pH	Ammonia N (mg/g)	Organic N (mg/g)
Mangoku-Ura (38°25'N, 141°24'E)	20 June 1974	2	21	Mud		0.071	7.8
Simoda Bay (34°40'N, 138°57'E)	8 August 1974	3	26	Sandy mud	7.4	0.028	0.56
Tokyo Bay (35°34'N, 139°53'E)	14 August 1974	18	20	Mud		0.25	3.6

TABLE 2. *Products of ^{15}N-labeled nitrate reduction in coastal sediments*[a]

Sediment origin	Production of		
	N_2[b]	NH_4^+[b]	PON[b]
Mangoku-Ura	2.93×10^{-2} (33%)[c]	4.62×10^{-2} (52%)	1.32×10^{-2} (15%)
Simoda Bay	1.78×10^{-2} (80%)	0.35×10^{-2} (16%)	0.09×10^{-2} (4%)
Tokyo Bay	0.84×10^{-2} (60%)	0.10×10^{-2} (7%)	0.46×10^{-2} (33%)

[a] Experimental conditions: (1) Mangoku-Ura sediment: 20°C, incubation time, 6 h; (2) Simoda Bay sediment: 26°C, incubation time, 8 h; (3) Tokyo Bay sediment: 20°C, incubation time, 10 h.

[b] μg atom of N per g per h.

[c] Numbers in parentheses represent the ratio of each product to the sum of three products.

TABLE 3. *Numbers of viable bacteria and denitrifying activity in Mangoku-Ura sediment*[a]

Determination	0 to 3 cm	18 to 20 cm
Heterotrophic bacteria (cells/g)	2×10^9	1×10^6
Nitrite-producing bacteria (cells/g)	7×10^5	3×10^4
Denitrifying bacteria (cells/g)	5×10^3	6×10^1
N_2 production[b] (μg atom of N per g per h)	2.9×10^{-2}	1.0×10^{-3}

[a] Sediment sample was collected on 20 June 1974 at the center of Mangoku-Ura.

[b] Denitrifying activity at 20°C in the presence of 30 μg atom of NO_3^--N per liter.

(1). Therefore, the rate of denitrification estimated only from N_2 production might be somewhat underestimated, but the error introduced by discounting nitrous oxide will probably be less than 10% (15).

Dissolved organic nitrogen is formed from ammonia inside the microbial cells and may be excreted and/or liberated from the cells after their death. During incubation periods of 10 h or less, its contribution would be unlikely to alter the nitrogen balance described in Table 2.

It is generally accepted that denitrification is dominant in soil systems when nitrate reduction proceeds under anaerobic conditions (4, 17). Only a minor portion of nitrate-N is recovered as oganic nitrogen. Working with lake sediments, Goering and Dugdale (5) have provided data supporting this view. Stanford et al. (14) showed, however, that the addition of glucose to soil greatly stimulates anaerobic ammonia formation from nitrate. Our results clearly demonstrate the simultaneous occurrence of denitrification and reduction of nitrate (nitrite) to ammonia and organic nitrogen in anaerobic marine coastal sediments. In Mangoku-Ura sediment, about 70% of the nitrate reduced is converted to these forms and recycled within the sediment ecosystem. Chen et al. (3) found that [^{15}N]ammonia is formed when lake sediments are incubated with [^{15}N]nitrate. Two pathways are postulated. One would require [^{15}N]ammonia production by decomposition of ^{15}N-labeled organic nitrogenous compounds or ammonification. The other entails excretion of [^{15}N]ammonia by nitrogen-fixing bacteria. In the latter, [^{15}N]N_2 yielded from [^{15}N]nitrate by denitrification would be fixed to produce [^{15}N]ammonia. The ammonia production is three to four times faster than that of PON production in Mangoku-Ura and Simoda Bay sediments (Table 2). Therefore, it is unlikely that ammonification forms a major route of ammonia formation in these sediments. The second pathway is also untenable. A preliminary observation showed that the bacterial fixation of nitrogen in Mangoku-Ura sediment is about one order of magnitude lower than that of denitrification. Even if we adopt the unrealistic assumption that [^{15}N]N_2 produced by denitrifiers is fixed by nitrogen-fixing bacteria and the [^{15}N]-ammonia yielded is excreted immediately into ambient water without dilution of ^{15}N, it is difficult to explain the observation that ammonia formation proceeds about two times faster than does denitrification (Table 2). Ammonia content in the sediments examined was high (Table 1). High concentration of ammonia represses the assimilatory but not the dissimilatory nitrate reduction (11). We conclude that ammonia is formed through a dissimilatory type of bacterial nitrate reduction in some marine coastal sediments. The possibility must be considered that enteric bacteria are responsible for this ammonia formation.

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